PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 15 May 2000 (15.05.00)	
International application No. PCT/US99/20349	**Applicant's or agent's file reference** MSK.P040WO
International filing date (day/month/year) 03 September 1999 (03.09.99)	**Priority date** (day/month/year) 04 September 1998 (04.09.98)
Applicant SADELAIN, Michel et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

31 March 2000 (31.03.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Pascal Piriou
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

COPY

European Patent Office

SUPPLEMENTARY EUROPEAN SEARCH REPORT

Application Number
EP 99 94 5508

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Y,D	ALVAREZ-VALLINA L ET AL: "ANTIGEN-SPECIFIC TARGETING OF CD28-MEDIATED T CELL CO-STIMULATION USING CHIMERIC SINGLE-CHAIN ANTIBODY VARIABLE FRAGMENT-CD28 RECEPTORS" EUROPEAN JOURNAL OF IMMUNOLOGY, WEINHEIM, DE, vol. 26, no. 10, 1 October 1996 (1996-10-01), pages 2304-2309, XP000646504 ISSN: 0014-2980 * the whole document *	1-10	C12N15/63 C07K14/705
Y	MORITZ D ET AL: "A SPACER REGION BETWEEN THE SINGLE CHAIN ANTIBODY AND THE CD3 KSI-CHAIN DOMAIN OF CHIMERIC T CELL RECEPTOR COMPONENTS IS REQUIRED FOR EFFICIENT LIGAND BINDING AND SIGNALING ACTIVITY" GENE THERAPY, MACMILLAN PRESS LTD., BASINGSTOKE, GB, vol. 2, no. 8, 1 October 1995 (1995-10-01), pages 539-546, XP000674790 ISSN: 0969-7128 * the whole document *	4	TECHNICAL FIELDS SEARCHED (Int.Cl.7) C07K
Y	TRAN A-C ET AL: "CHIMERIC DZETA-RECEPTORS DIRECT HUMAN NATURAL KILLER (NK) EFFECTOR FUNCTION TO PERMIT KILLING OF NK-RESISTANT TUMOR CELLS AND HIV-INFECTED T LYMPHOCYTES" JOURNAL OF IMMUNOLOGY, THE WILLIAMS AND WILKINS CO. BALTIMORE, US, vol. 155, no. 2, June 1995 (1995-06), pages 1000-1009, XP000882943 ISSN: 0022-1767 * the whole document *	1-10	

The supplementary search report has been based on the last set of claims valid and available at the start of the search.

Place of search	Date of completion of the search	Examiner
MUNICH	3 July 2002	Wimmer, G

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C04)

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference MSK.P040WO	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US99/20349	International filing date *(day/month/year)* 03 SEPTEMBER 1999	Priority date *(day/month/year)* 04 SEPTEMBER 1998
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand 31 MARCH 2000	Date of completion of this report 15 JANUARY 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Dorothea Lawrence for JILL D. MARTIN Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet) (July 1998)★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/20349

I. Basis of the report

1. With regard to the ~~elements~~ of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-15 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the claims:
pages 16-18 , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the drawings:
pages 1-3 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the sequence listing part of the description:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/20349

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	1-16	YES
	Claims	NONE	NO
Inventive Step (IS)	Claims	1-16	YES
	Claims	NONE	NO
Industrial Applicability (IA)	Claims	1-16	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 1-16 meet the criteria set out in PCT Article 33(2)-(4), because the prior art does not teach or fairly suggest the claimed invention.

Applicants' arguments are persuasive in that the combined cited prior art fail to provide a basis for an assertion of lack of novelty or lack of an inventive step.

NEW CITATIONS

DARCY et al. Expression of cytotoxic T lymphocytes of a single-chain anti-carcinoembryonic antigen antibody. Redirected Fas Ligand-mediated lysis of colon carcinoma. European Journal of Immunology. May 1998, Vol. 28, No. 5, pages 1663-1672, see entire document.

FITZER-ATTAS et al. Harnessing Syk family tyrosine kinases as signaling domains for chimeric single chain of the variable domain receptors: optimal design for T cell activation. Journal of Immunology. January 1998, Vol. 160, No. 1, pages 145-154, see entire document.

GALLARDO et al. Recombinant retroviruses pseudotyped with the vesicular stomatitis virus glycoprotein mediate both stable gene transfer and pseudotransduction in human peripheral blood lymphocytes. Blood. August 1997, Vol. 90, No. 3, pages 952-957, see entire document.

LIU et al. Identification of rat prostatic steroid-binding protein as a target antigen of experimental autoimmune prostatitis. Implications for Prostate Cancer Therapy. Journal of Immunology. 1997, Vol. 159, No. 1, pages472-480, see entire document.

XUE et al. Induction of human cytotoxic T lymphocytes specific for prostate-specific antigen. Prostate. February 1997,Vol.30,No. 2, pages 73-78, see entire document.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

CLASSIFICATION:

The International Patent Classification (IPC) and/or the National classification are as listed below:

IPC(7): C12N 15/63+; A61K 39/21, 48/00; C07K 14/00 and US Cl.: 435/320.1; 514/44; 424/188.1; 424/93.6; 530/324

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/20349

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 15/63+; A61K 48/00, 39/21, 48/00; C07K 14/00+

US CL : 435/320.1; 514/44; 424/188.1; 424/93.6; 530/324

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/320.1; 514/44; 424/188.1; 424/93.6; 530/324

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN, Caplus, Biosis, Medline, WEST, dialog

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SALGALLER et al. Report of immune monitoring of prostate cancer patients undergoing T-cell therapy using dendritic cells pulsed with HLA-A2-specific peptides from prostate-specific membrane antigen (PSMA). Prostate. May 1998, Vol. 35, No. 2, pages 144-151, see entire document.	1-6, 12-13
Y	MURPHY et al. Phase I clinical trial: T-cell therapy for prostate cancer using autologous dendritic cells pulsed with HLA-A0201-specific peptides from prostate-specific membrane antigen. The Prostate. 1996, Vol. 29, No. 6, pages 371-380, see entire document.	1-6,12-13

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 29 DECEMBER 1999	Date of mailing of the international search report 03 FEB 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer GAI (JENNIFER) MI LEE Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

PATENT COOPERATION TREATY

REPORTING LETTER
COMPUTER DOCKET
PAPER DOCKET

PCT

From the INTERNATIONAL BUREAU

To:
LARSON, Marina, T.
Oppedahl & Larson LLP
P.O. Box 5270
Frisco, CO 80443-5270
ÉTATS-UNIS D'AMÉRIQUE

RECEIVED
MAR 29 2000
S&H

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

Date of mailing (day/month/year) 16 March 2000 (16.03.00)	
Applicant's or agent's file reference MSK.P040WO	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/US99/20349	03 September 1999 (03.09.99)	04 September 1998 (04.09.98)

Applicant
SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   EP,JP,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   CA

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   16 March 2000 (16.03.00) under No. WO 00/14257

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer J. Zahra
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

M

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
MARINA T. LARSON
OPPEDAHL & LARSON LLP
PO BOX 5270
FRISCO, CO 80443 5270

PCT

NOTIFICATION OF RECEIPT
OF DEMAND BY COMPETENT INTERNATIONAL
PRELIMINARY EXAMINING AUTHORITY

(PCT Rules 59.3(e) and 61.1(b), first sentence
and Administrative Instructions, Section 601(a))

Date of mailing *(day/month/year)*	01 MAY 2000
Applicant's or agent's file reference: MSK.P040WO	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/20349	03 SEP 99	04 SEP 98

Applicant
SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH

1. The applicant is hereby notified that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

   31 MARCH 2000

2. That date of receipt is:

   [X] the actual date of receipt of the demand by this Authority (Rule 61.1(b)).

   [ ] the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).

   [ ] the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. [ ] ATTENTION: That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the election(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide*, Volume II.

   [ ] *(If applicable)* This notification confirms the information given by telephone, facsimile transmission or in person on:

   _____

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/ Assistant Commissioner for Patent Box PCT Washington, D.C. 20231 Attn:RO/US Facsimile No. 703-305-3230	Authorized officer Misty Walker Stacia Cadmus for Telephone No. 703-305-3682

Form PCT/IPEA/402 (July 1998)

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: MARINA T. LARSON
OPPEDAHL & LARSON LLP
PO BOX 5270
FRISCO, CO 80443-5270

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing *(day/month/year)*	01 FEB 2001
Applicant's or agent's file reference MSK.P040WO	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/US99/20349	03 SEPTEMBER 1999	04 SEPTEMBER 1998

Applicant
SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

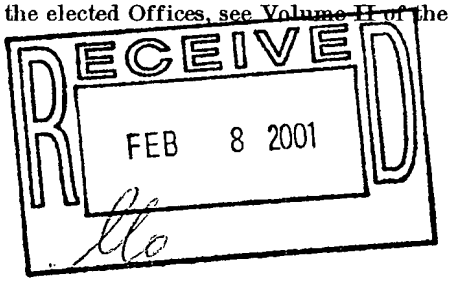
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.



Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JILL D. MARTIN Telephone No. (703) 308-0196

Form PCT/IPEA/416 (July 1992)★

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/63, A61K 48/00, 39/21, 48/00, C07K 14/00

A1

(11) International Publication Number: WO 00/14257

(43) International Publication Date: 16 March 2000 (16.03.00)

(21) International Application Number: PCT/US99/20349

(22) International Filing Date: 3 September 1999 (03.09.99)

(30) Priority Data:
60/099,138 4 September 1998 (04.09.98) US

(71) Applicant *(for all designated States except US)*: SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH [US/US]; 1275 York Avenue, New York, NY 10021 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: SADELAIN, Michel [CA/US]; 401 E 89th Street #9K, New York, NY 10128 (US). BANDER, Neil, H. [US/US]; 2 Hemlock Hill, Chappaqua, NY 10128 (US). GONG, Michael [US/US]; 1233 York Avenue #15N, New York, NY 10021 (US).

(74) Agent: LARSON, Marina, T.; Oppedahl & Larson LLP, P.O. Box 5270, Frisco, CO 80443-5270 (US).

(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: FUSION RECEPTORS SPECIFIC FOR PROSTATE-SPECIFIC MEMBRANE ANTIGEN AND USES THEREOF

(57) Abstract

A fusion receptor composition which is effective to promote a cellular immune response to prostate-specific membrane antigen (PSMA) *in vivo* when the fusion receptors is expressed by T lymphocytes has the structure: PSMA-scFv: connector: cytoplasmic domain. The PSMA-scFv in this structure is a single chain antibody cloned from the V region genes of a hybridoma specific for PSMA. The connector region is provided to give a spacing between the OSMA-scFv and the cytoplasmic domain, such that both can retain substantial function. A suitable connector is the CD8 hinge, although other connectors of greater or lesser length might be used. The cytoplasmic domain is included to direct the function of the fusion receptor. One exemplary cytoplasmic domain which can be used in the fusion receptor of the invention is a T cell receptor ζ-chain cytoplasmic domain. An expression vector encoding the fusion receptor is transduced into primary T lymphocytes obtained from an individual to be treated. The transduced lymphocytes are returned to the patient where cells expressing the fusion receptor secrete interleukin 2 and proliferate in response to PSMA-positive cells. The resulting cytotoxic lymphocytes specifically lyse cells expressing PSMA and thus can be used to target PSMA-positive tumor cells and neovasculature.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
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DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

FUSION RECEPTORS SPECIFIC FOR PROSTATE-SPECIFIC MEMBRANE ANTIGEN AND USES THEREOF

This application claims priority from US Provisional Application No. 60/099,138, filed September 4, 1998, which for purposes of the United States and countries allowing such incorporation, is incorporated herein by reference.

Field of the Invention

This invention relates to fusion receptors for prostate-specific membrane antigen (PSMA), and to uses thereof in the treatment of prostate cancer, other cancers expressing PSMA and tumor neovasculature. The inventions provides fusion receptors, nucleic acids encoding these fusion receptors, and transduced cells expressing the fusion receptors, as well as methods of using the transduced cells.

Background of the Invention

A long-standing goal of cancer research has been to stimulate the immunological rejection of tumors. This goal is based on the hypothesis that many tumors express foreign or mutated forms of antigens that can potentially serve as targets for their destruction by the immune system. Cellular immunity plays the key role in this rejection, with both T helper cells and cytolytic T lymphocytes (CTLs) being involved (Greenberg, *Adv. Immunol.* 49: 281-355 (1991).

There are several reasons why even those tumors that express rejection antigens can evade destruction by T cell immunity. Destruction of immunological targets requires T lymphocyte recognition via the T cell receptor (TCR) of antigenic peptides presented in the context of major histocompatibility complex (MHC) molecules on antigen-presenting cells (APC). Some tumors fail adequately to process and present antigens to T cells because of reduced expression of MHC class I molecules.

Many strategies have been devised to render tumor cells more immunogenic. One is based on the genetic engineering of tumor cells to stimulate the generation of tumor-specific effector T cells *in vivo*. This has been investigated by direct MHC class I gene transfection to enhance expression of MHC by introduction of the γ-interferon cDNA to

upregulate endogenous class I antigens or by tranfecting tumor cells with cytokines with the hope that interleukin paracrine secretion of lymphokines can substitute for T cell help, induce tumor specific cytotoxic T lymphocytes, and cause tumor rejection.

The molecular basis of T cell costimulation results from an interaction of the T cell surface receptor CD28 with the costimulatory ligand B7, which is primarily expressed on the surface of professional APCs and activated B cells leading to IL-2 secretion and clonal expansion of the activated T cells. *In vitro* and *in vivo* studies showed that signals transduced by the CD28 receptor determine whether TCR occupancy results in a productive immune response or clonal anergy. Therefore, one factor accounting for the poor immunogenicity of MHC-expressing tumors is that, despite presentation of potentially immunogenic peptides in the context of MHC molecules, tumors lack the costimulatory molecule B7, and thus fail to elicit a full activation of T cells and therefore an effective anti-tumor T cell response. Thus, the introduction of the B7 molecule (CD28 ligand) in tumor cells is one discussed therapy today (melanoma: Townsend et al, 1993; Chen et al, 1992; colon carcinoma: Townsend et al, 1994) to provide protective immunity by autologous $CD8^+$ T cells which leads to a potent rejection of modified and unmodified tumor cells in vivo. $CD4^+$ and $CD8^+$ immunity induced by immunization with class $II^+B7\text{-}1^+$-transfected sarcoma cells are also widely discussed immunotherapy strategies.

Another approach is based on the manipulation of the effector cells, i.e., T lymphocytes, rather than the antigen-presenting cells or tumor cells. T cells can recognize and lyse tumor cells provided that they bind to the tumor cells and are appropriately activated. T cell activation operates according to the two signal model, which states that lymphocytes require for optimal activation both an antigen-specific signal delivered through the antigen receptor and a second antigen nonspecific or costimulatory signal. T cell costimulatory pathways determine whether TCR complex engagement results in functional activation or clonal anergy of $CD4^+$ T cells.

One means of generating tumor-specific T lymphocytes is their modification by gene transfer of tumor-specific fusion molecules. The introduction of chimeric molecules in T cells combining tumor specific single chain variable fragment (scFv) with signal transduction domains of TCR related activation molecules is reported by a number of groups

(Eshar et al, *Springer Semin. Immunopathol.* 18: 199-209 (1993)). These genetically modified T cells are able to target tumor cells and to destroy them *in vitro*, but based on the two signal model for T cell activation, the reinfusion of these transduced T lymphocytes is limited by the incomplete activation signal after antigen recognition and clonal expansion *in vivo* is not successful.

Alvarez-Vallina et al., *Eur. J. Immunol.* 26: 2304-2309 (1996) have shown that antigen dependent IL-2 secretion can be stimulated *in vitro* in Jurkat cells expressing a chimeric molecule formed from an antigen-specific single chain antibody variable fragment (scFv) and a truncated CD28 (amino acids 124-202; transmembrane and cytoplasmic domains), when the cells are exposed to the antigen in the presence of anti-CD3 or ionomycin as a costimulatory signal. Similarly, antigen dependent IL-2 secretion can be stimulated *in vitro* in Jurkat cells expressing a chimeric molecule formed from an antigen-specific scFv and the ζ-chain of CD3, when the cells are exposed to the antigen in the presence of anti-CD28 or ionomycin as a costimulatory signal. Cells expressing both chimeric molecules displayed responses to either antigen in the presence of appropriate costimulatory molecules. Alvarez-Vallina et al. suggests that these results offer the possibility that addition of antigen-specific CD28 mediated signaling could improve adoptive immunotherapies.

It is a substantial step, however, from such *in vitro* results to therapeutic efficacy *in vivo*. Importantly, while IL-2 secretion is suggestive of T cell activation, it can frequently be followed by T cell anergy or apoptosis (presumably as a result of inadequate costimulation) which results in the death of the T cells rather than the development of an immune response. There is no assurance that signaling species will be present *in vivo* to fulfill the role of the external costimulatory signals supplied by Alvarez-Vallina et al. in their *in vitro* experiments, or that these signals will result in the level of clonal expansion which is required to mount and maintain a therapeutically meaningful cytotoxic T cell response. Furthermore, artificial T-cell receptors have not been shown to function in human peripheral blood lymphocytes (PBL) and in particular in the T cells of actual cancer patients. The applicability of findings in normal T cells or carefully selected leukemic cells lines to T cells from cancer patients cannot be taken for granted given the signaling defects frequently observed in cancer patients and chronically tumor-bearing mice. Mizoguchi et al., *Science*

258: 1795-1798 (1992); Ochoa et al., in *Important Advances in Oncology*, J.B. Lippincott Co., Philadelphia (1995); Zier et al., *Hum. Gene Ther.* 6: 1259-1264 (1995). Some of these defects, which include ζ-chain, lck and ZAP-80 abnormalities, could limit the function of artificial TCRs in cancer patient T cells. Thus, there remains a need for a method for sustaining the formation of tumor-specific T lymphocytes which can be successfully reintroduced into a host organism, preferably a human being, for the *in vivo* generation of a cellular immune response to the tumor.

It is an object of the present invention to provide such a method, and to provide fusion proteins, and nucleic acid constructs encoding such fusion proteins which can be used in such a method.

It is a further object to provide such methods and fusion proteins specific for prostate-specific membrane antigen.

Summary of the Invention

The present invention provides a fusion receptor composition which is effective to promote a cellular immune response to a target antigen *in vivo* when the fusion receptor is expressed by T lymphocytes. By way of example, when the target antigen is prostate-specific membrane antigen (PSMA), the fusion receptor has the structure:

PSMA-scFv : optional connector : cytoplasmic domain.

The PSMA-scFv in this structure is a single chain antibody cloned from the V region genes of a hybridoma specific for PSMA. The optional connector region is provided to give a spacing between the PSMA-scFv and the cytoplasmic domain, such that both can retain substantial function. In cases where a connector is needed, a suitable connector is the CD8 hinge, although other connectors of greater or lesser length might be used. The cytoplasmic domain is included to direct the function of the fusion receptor. One exemplary cytoplasmic domain which can be used in the fusion receptor of the invention is a T cell receptor ζ-chain cytoplasmic domain.

In accordance with the method of the invention, an expression vector encoding the fusion receptor is transduced into primary T lymphocytes obtained from an individual to be treated, for example an expression vector encoding the PSMA-scFv containing fusion

receptor is suitably transduced into cells from a human patient who has been diagnosed with prostate cancer. The transduced lymphocytes are returned to the patient where cells expressing the fusion receptor secrete interleukin 2 and proliferate in response to PSMA-positive cells. The resulting cytotoxic lymphocytes specifically lyse cells expressing PSMA and thus can be used to target PSMA-positive tumor cells. Expression of the fusion receptor in CD4+ or CD8+ T-cells, natural killer (NK) cells or other immune effector cells allows these cells to target any tissue (including tumor tissue) expressing PSMA. Thus, such cells can be used to treat prostate cancer, other cancers expressing PSMA and tumor-associated neovasculature.

Brief Description of the Drawings

Fig. 1 shows the structure of a retroviral vector Pz-1 including a gene for a PSMA-specific fusion receptor in accordance with the invention;

Figs. 2 A-E show cytotoxicity of Pz-1 transduced PBL with respect to various target cells;

Fig. 3 shows the time course for cocultivation of transduced T cells with fibroblasts;

Fig. 4A shows T cells proliferation in coculture with various types of fibroblast cells;

Fig. 4B shows cell lysis by T cells after prior exposure to coculture conditions; and

Fig. 5 shows IL-2 production by transduced T cells in coculture with various types of fibroblast cells.

Detailed Description of the Invention

In a first aspect, the present invention provides fusion receptors which are useful in the generation of a cellular immune response to cells which express PSMA. Such fusion receptors have the general structure:

PSMA-scFv : optional connector : cytoplasmic domain.

This structure is produced by expression in transduced cells of a DNA sequence encoding the amino acid sequence of the fusion receptor.

In the general formula set forth above, PSMA-scFv is a single chain antibody cloned from the V region genes of a hybridoma specific for PSMA. A suitable hybridoma for this purpose is J591, which is described in Liu et al., *Cancer Res.* 57: 3629-3635 (1997), although other hybridomas which produce monoclonal antibodies specific to PSMA could also be employed. The production of such hybridomas has become routine, and the procedure will not be repeated here.

A technique which can be used for cloning the variable region heavy chain (V_H) and variable region light chain (V_L) has been described in Orlandi et al., *Proc. Natl Acad. Sci. (USA)* 86: 3833-3837 (1989). Briefly, mRNA is isolated from the hybridoma cell line, and reverse transcribed into complementary DNA (cDNA), for example using a reverse transcriptase polymerase chain reaction (RT-PCR) kit. Sequence-specific primers corresponding to the sequence of the V_H and V_L genes were used, for example Seq. ID. Nos. 1-4. Sequence analysis of the cloned products and comparison to the known for the V_H and V_L genes showed that the cloned V_H gene matched expectations. For the V_L gene, however, the cloned sequence did not match expectations, and contained a stop codon in the anticipated open reading frame. To correct this, corrective primers (Seq. ID. Nos. 5 and 6), containing inserted bases to correct the differences between the clone and the known sequence were used to amplify the cloned V_L gene to produce polynucleotides encoding the V_L gene.

The cytoplasmic domain portion of the general formula set forth above is selected to enhance the characteristics of the fusion receptor for purposes of promoting a cellular immune response to the antigen recognized by the scFv portion of the fusion receptor. In general, the cytoplasmic domain is the cytoplasmic domain of a molecule which functions as a transducer of a mammalian immune response in the presence of an MHC-peptide complex or costimulatory factor. Representative, non-limiting examples of cytoplasmic domains which may be employed in the present invention include the ζ-chain cytoplasmic domain, the CD28 cytoplasmic domain (particularly a fragment spanning amino acids 336 to 663 of CD28 cDNA), 41BB, CD40, ICOS and trance.

When the cytoplasmic domain is the ζ-chain derived the TCR complex, the fusion receptor of the invention closely mimics a native TCR. In this case, it might be expected that binding of an antigen to the scFv portion of the fusion receptor would result in changes to the ζ-chain which would trigger an intracellular phosphorylation cascade comparable to that observed when an antigen bound to an APC interacts with a native TCR. As noted above, however, this signal transduction is not sufficient to produce a complete cellular immune response, and a secondary signal must me provided to avoid anergy and premature cell death through apoptosis. In the art, this secondary signal was provided by a signaling antibody unsuited for use *in vivo*. The present invention provides for the first time the understanding that cells expressing PSMA and a costimulatory signal such as B7.1 provide sufficient secondary signal to maintain a stable population of PSMA-targeting T cells when the cells are transduced with the fusion protein of the invention.

Instead to the ζ-chain, the fusion receptors of the invention may include other cytoplasmic domains. For example, CD28 can be used as the cytoplasmic domain to enhance T-cell activation, survival and proliferation. A preferred CD28 moiety is one which spans amino acids 336 to 663 of CD28 cDNA, in which case no connector is needed to retain functon. PSMA-fusion receptors incorporating 41BB as the cytoplasmic domain have also been prepared. Both the PSMA-CD28 and the PSMA-41BB fusion receptors have been made and tested in the same experimental model used with the PSMA-ζ chain fusion receptor. In both cases, sustained proliferation was observed in both human $CD4^-$ and $CD8^-$ primary T cells (PBL) in the presence of $PSMA^+$ cells, with more sustained proliferation being provided by the PSMA-41BB fusion receptor. High production of IFN-γ and IL-2 was observed, for PSMA-41BB and PSMA-CD28 transduced, respectively. In each of the experiments performed, an external signal to complement the signaling of the fusion receptor was used. However, transfection of the PBL with fusion receptors encoding both the ζ-chain and either CD28 or 41BB or a comparable costimulatory molecule would eliminate this requirement. Thus, for example, PBL transduced with both the PSMA-ζ chain fusion recpetor and either a PSMA-fusion receptor with a secondary signaling moiety would provide therapeutic efficacy for *in vivo* use.

Between the scFv and the cytoplasmic domain may be a connector. The function of the connector is to act as a spacer so that both the scFv and the cytoplasmic domain can be functionally oriented within the membrane of the transduced cell. One exemplary connector is the CD8 hinge, although other connectors of greater or lesser length could be used. In some cases, such as using the CD28 fragment described herein, no connector is required to permit the molecules to assume the desired orientation.

The chimeric fusion receptors are introduced into the individual to be treated (preferably a human) in one of two ways. Gene transfer can be carried out into bone marrow cells, either *in vivo* or *ex vivo*, or into immune effector/inflammatory cells such as T-lymphocytes or NK cells. Gene transfer may also be carried out into antigen presenting cells, particularly dendritic cells. In the case of dendritic cells, CD40 and trance are the preferred cytoplasmic domain.

A preferred approach to this gene transfer is using retroviral vectors encoding the fusion receptor. A particularly preferred approach utilizes an SFG retroviral vector (Riviere et al., *Proc. Natl Acad Sci. (USA)* 92: 6733-6737 (1995)) transduced into patient PBL using gibbon ape leukemia virus (GALV) envelope-pseudotyped virions. (Gallardo et al., *Blood* 90: 952-957 (1997).

The PSMA-specific fusion receptor of the present invention is useful in the treatment of prostate cancer. In addition, because PSMA is also found in the neovasculature of renal cell, urothelial, colon, breast and lung carcinomas, melanomas and some sarcomas, the PSMA-specific fusion receptor of the invention has broader applicability. Thus, in one aspect of the invention, the present application describes a method for treatment of cancers in which the cancer cells or neovasculature are characterized by expression of PSMA, comprising administering to a patient suffering from such a cancer patient-derived lymphocytes which express with a PSMA fusion receptor having the structure

PSMA-scFv : optional connector : cytoplasmic domain.

As used herein, the concept of "administration" is intended to encompass both *in vivo* methods, in which the fusion receptor is introduced into the lymphocytes without first removing them from the patient, and *ex vivo* methods where the patient-derived lymphocytes

are obtained from the patient, transduced with the PSMA-specific fusion receptor and then reintroduced to the patient.

The transduced lymphocytes are introduced in an amount to provide therapeutic benefit. Where sufficient clonal expansion of the transduced lymphocytes occurs *in vivo*, a long-term immunity to the tumor cells may be induced after a single administration. If the transduced lymphocytes are less stable, multiple infusions may be required to obtain remission of a particular cancer, and long-term protection may not be achieved. In either case, the determination of the appropriate therapeutic regimen is a matter of routine developed in the course of clinical trials.

The invention will now be further described and illustrated with reference to the following non-limiting examples.

Example 1

PSMA-scFv was created by cloning the immunoglobulin genes from the J591 hybridoma encoding the variable region of the heavy chain (V_H and the variable region of the light chain (V_L). The V_H and V_L genes were cloned using the technique previously described by Orlandi et al., *supra*. Briefly, mRNA was isolated from the J591 hybridoma cell line and reverse transcribed into cDNA using a reverse transcriptase polymerase chain reaction (RT-PCR) kit obtained from Pharmacia, Pisacatway, NJ. The V_H and V_L genes were cloned from the cDNA using the following degenerate primers:

V_H backward:
AGGTSMARCTGCAGSAGTCWGG Seq. ID. No. 1

V_H forward:
TGAGGAGACGGTGACCGTGGTCCCTTGGCCCCAG Seq. ID. No. 2

V_L backward:
GACATTGAGCTCACCCAGTCTCCA Seq. ID. No. 3

V_L forward:

TGCGGCCGCCCGTTTGATCTCCAGCTTGGTCCC Seq. ID. No. 4

Sequence analysis of the V_H gene confirmed an appropriate open reading frame. However, the V_L gene sequence analysis revealed a stop codon in the anticipated reading frame. The sequences of the genes encoding the J591 monoclonal antibody heavy and light chain were compared to the sequences of the cloned products, and several discrepancies were noted between the V_L sequences. The major difference was that the primer pair used deleted a nucleotide from the actual sequence, resulting in an open reading frame shift that produced a stop codon. Nucleotide sequence corrections in the V_L product were made using corrective primers based on the actual sequences and using these primers in a second PCR amplification utilizing the obtained V_L sequence as a template. The corrective primers were:

V_L backward:

GAAGAAGATC***T***G***A***CATTGTGATGACCAGTCTC***A***CAAATTCATG Seq. ID. No. 5

V_L forward:

TGCGGCCGCCCGTTT***C***A***GG***TCC***A***GCATGGTCCCAGCACCG Seq. ID. No. 6

wherein the bold and italicized letters indicate additions/substitutions to correct the obtained V_L sequence to the actual J591 V_L sequence.

Next, an oligonucleotide encoding the human CD8 leader sequence was cloned to the 5'-end of the V_H gene, and the 3'-end of the V_H gene was cloned to an oligonucleotide encoding a $(gly\text{-}ser_2)_5$ linker followed by the V_L gene, creating the PSMA-specific scFv. The scFv was then cloned to the CD8 hinge and transmembrane domains, followed by the T cell receptor ζ-chain cytoplasmic domain to create Pz-1, a PSMA-specific scFv/ζ-chain chimeric T-cell receptor. The Pz-1 fusion gene was then cloned into the SFG retroviral vector (Riviere et al, *supra*) as illustrated in Fig. 1.

Example 2

The SFG retroviral vector containing Pz-1 was transduced into PBL's harvested from five human patients suffering from prostate cancer with a variety of clinical stages using GALV envelope pseudotyped virions as previously described. Gallardo et al., *supra*. The clinical status of three representative patients is summarized in Table 1.

Table 1

#	age	time since dx (mon)	GG	stage at dx	treatment	current stage	current PSA (ng/ml)
1	67	19	3+3	T1c	none	T1c	10.2
2	85	251	5+4	T3	hormonal	T4M+	501.2
3	54	7	4+5	T1c	RRP	pT3CNoMx rising PSA	4.0

In Table 1, the age is the current age of the patient, the time since dx is the time elapsed between date of diagnosis of prostate cancer and PBL harvest; GG is the Gleason grade of the patient's most recent prostate cancer pathology; stage at dx is the clinical stage at the time of original diagnosis; RRP in the treatment column stands for radical retropubic prostatectomy; current stage is the current clinical or pathological state and current PSA is the most recent serum prostate specific antigen (PSA) level. Normal PSA is in the range of 0-4 ng/ml. A rising PSA following RRP is biochemical evidence of residual prostate cancer, although the site of the residual disease is unknown.

After retroviral infection, the PBL were expanded 4 to 14 days in the presence of interleukin-2 (IL-2). Gene transfer efficiency was monitored by FACS analysis using a FITC-conjugated Pz-1 idiotype-specific antiserum. After incubation with the FITC labeled antiserum, the cells were washed incubated with 10% normal mouse serum, and stained with a PE-conjugated anti-CD8 mAb. The gene transfer efficiency observed varied between 20% and 50% in both $CD8^+$ and $CD4^+$ cells for Pz-1 and controls.

Example 3

Cytotoxic T lymphocyte (CTL) assays were performed on the human prostate cancer cell line LNCaP which abundantly expresses PSMA. In order to confirm that the

cytotoxicity was PSMA-specific, PSMA was expressed in PC-3, a PSMA-negative human prostate cancer cell line and EL-4, a murine thymoma cell line. PBL from the three patients of Table 1 were transduced with either Pz-1 or NTP, a mutated human low-affinity nerve growth factor receptor used as a control cell surface marker. (Gallardo et al., *supra*). Transduction efficiency (%TR) measured as described above, and the fraction of $CD8^+$ and $CD56^+$ cells on the day of the CTL assay are reported in the common legend of Fig. 2. The cultured PBL, harvested 4 to 14 days after transduction, were incubated for 4 hours at different effector to target (E:T) rations with 10^4 ^{51}Cr-labeled target cells. Percent specific lysis was determined for $PSMA^+$ LNCaP cells(Fig. 2A), PC3 cells transduced with PSMA (Fig. 2B), wild-type, $PSMA^-$ - PC3 cells (Fig. 2C); EL4 cells transduced with PSMA (Fig. 2D) and wild-type $PSMA^-$ - EL4 cells (Fig. 2E). As shown, for all patients, PBL transduced with Pz-1 but not NTP effectively lysed $PSMA^+$ targets. The same results were obtained using Pz-1 transduced PBL from two other patients with advanced, hormone refractory prostate cancer. The level of cytolytic activity did not closely correlate with the transduction efficiency achieve din each case. Background was variable on human target cells and uniformly low on murine cells. T cells transduced with an irrelevant scFv-ζ chain fusion receptor did not lyse target cells expressing PSMA above background levels. Thus, elevated PSMA-specific cytotoxicity was obtained in Pz-1 transduced T cells derived from five out of five prostate cancer patients tested, independent of their age or clinical stage of disease.

Example 4

To further assess the response of Pz-1 transduced primary T cells to PSMA, we investigated whether $Pz\text{-}1^+$ PBL could undergo proliferation upon engagement with cell-bound PSMA and sustain thereafter their cytolytic potential. A cocultivation system was established in which transduced T cells were cultured with a layer of irradiated NIH3T3 fibroblasts expressing various combinations of PSMA and B7.1 for four days with periodic sampling to measure levels of IL-2 as illustrated in Fig. 3. FACS cell counts using FITC-conjugated Pz-1 idiotype specific antibody and with either anti-CD4 or anti-CD8 were performed after four days of co-cultivation, and again 4 days later. The transduced T cell count was derived by multiplying the percentage of $CD4^+Pz\text{-}1^+$ or $CD8^+Pz\text{-}1^+$ double positive

cells by the number of viable cells. The results are shown in Fig. 4A, where /B7+PSMA, /PSMA and /B7 refer to the molecules expressed by the fibroblast layer.

PSMA induced proliferation of Pz-1 transduced T-cells, increasing the number of cells 6-8 fold after 4 days, and these transduced cells destroyed $PSMA^+$ fibroblast layers within 48 hours, while the $PSMA^-$ layers remained intact during the entire 4-day cocultivation. By day 8, however, the absolute number of $Pz\text{-}1^+$ cells dropped to 2-3 fold above initial levels. To address whether costimulation could amplify proliferation, B7.1 (CD80) was transduced into $PSMA^+$ and $PSMA^-$ fibroblasts. After 8 days of cocultivation, $Pz\text{-}1^+$ T cell counts were 5-8 fold higher in cultures with $PSMA^+$ B7.1 fibroblasts than with $PSMA^+B7.1^-$ fibroblasts, and 25-36 fold higher than in cultures with $PSMA^-$ $B7.1^+$ fibroblasts, suggesting that Pz-1 signaling synergized with the B7.1 mediated costimulation. Fibroblasts expressing PSMA± B7.1 did no induce proliferation of T cells transduced with an irrelevant scFv-ζ chain fusion receptor.

Supernatants of the cocultured T cells were sampled after 24, 48 and 72 hours and assayed for IL-2 and interferon-γ (IFN-γ) secretion. Pz-1 transduced cells released significant amounts of IL2 (Fig. 4B) and IFN-γ 24 hours after exposure to $PSMA^+$ fibroblasts. IL-2 was barely detectable in the supernatants of $Pz\text{-}1^+$ cells cocultured with NIH3T3 fibroblasts expressing only B7.1, or in the absence of any feeder layer, nor was it detectable in any coculture condition with T cells transduced with a control irrelevant scFv-ζ chain fusion receptor. However, IL-2 and IFN-γ production was strongly potentiated in the presence of PSMA and B7.1, resulting in 10-fold higher IL-2 release after 24 hours (Fig. 4B). This confirms that B7.1-mediated signaling provided functional costimulation in Pz-1 transduced cells.

Example 5

To test whether cytotoxic T cells retain their cytotoxic potential after restimulation with antigen, T cells were harvested 12-17 days after the start of coculture with fibroblast monolayers expressing PSMA and B7.1 and retested in the CTL assay. As shown in Fig. 5, expanded Pz-1 transduced T cells remained fully capable of lysing $PSMA^-$ target cells. Furthermore, the expanded $Pz\text{-}1^+$ cells were capable of a second round of proliferation

and IL-2 and IFN-γ secretion when reexposed to $PSMA^+B7.1^+$ fibroblasts. These findings indicate that at least a subset of the T cells transduced with the Pz-1 fusion receptor do no fatally undergo activation-induced cell death (AICD) or anergy, as they remain capable of proliferating and killing after contact with antigen. However, in the absence of costimulation, their proliferative potential appears limited.

These results are of critical importance to the utility of the invention in therapeutic applications. The acquisition of specific tumoricidal properties by autologous T cell is of only marginal importance if these cells are not able to carry out more than one cytotoxic hit. Indeed, physiological TCR engagement with peptide-MHC complexes result s in T cell activation which, in the absence of costimulation does not result in complete activation and can eventually lead to anergy or apoptosis. This is especially the case in previously activated cells, such as T cells, cultured for the purpose of clonal expansion or retroviral transduction. Restimulation of antigen-specific $CD4^+$ clones following their adoptive transfer in tumor-bearing animals has been suggested to cause clonal anergy.. In the case of T cells bearing artificial TCRs, the prospect of apoptotic cell death or anergy upon restimulation could be compounded by partial T cell activation if chimeric receptors fail to adequately recruit downstream signaling molecules. Faulty T cell activation could result in the induction of immune tolerance and the neutralization of the infused effector T cells. Such phenomenon could in part explain *in vivo* findings obtained with T cells expressing an ErbB-2 specific-ζ chain fusion receptor, required repeated high dose intra-tumoral administration to effectively eliminate established tumors. Alternschmidt et al., *J. Immunol.* 159: 5509-5515 (1997). The proliferation and cytokine release induced by $PSMA^+$ murine fibroblasts together with the cytotoxicity achieved against $PSMA^+$ murine EL4 cells suggest that the Pz-1 fusion receptor alone provides a sufficient signal to trigger T cell activation. However, although TCR signaling alone can induce lymphocyte proliferation, complete and more sustained activation generally requires that a costimulatory signal be provided in conjunction with TCR stimulation. The results of examples 1-5 show that Pz-1 receptor function is enhanced by costimulation and that costimulation can be provided by B7.1 expression on $PSMA^+$ target cells.

Example 6

In order to prepare a fusion receptor in which the cytoplasmic domain is derived from CD-28, the procedure of Example 1 was repeated only using a CD28 fragment in place of the ζ-chain segment. The CD28 cDNA fragment was obtained as follow.

A segment of the human CD28 cDNA that encodes part of the extracellular, the transmembrane, and the cytoplasmic domains (amino acids 336 to 663) was amplified by PCR from the plasmid pbsCD28, using the upstream primer

5'GCGGCCGCAATTGAAGTTATGTATCCT SEQ ID No. 7

and the downstream primer

5'TCGAGGATCTTGTCAGGAGCGATAGGCTGC SEQ ID No. 8.

These primers contain NotI and BamHI sites respectively for the insertion of the PCR product in the retroviral Vector SFG. Following digestion of the purified PCR product with NotI and BamHI, the CD28 fragment was ligated into the NotI an BamHI sites of the retroviral vector SFG, containing the CD8α leader sequence, followed by the single chain gene, encoding the V_H and V_L domains of the PSMA-specific antibody

CLAIMS

1. A fusion receptor composition having the structure:

PSMA-scFv : optional connector : cytoplasmic domain,

wherein PSMA-scFv represents a single chain antibody cloned from the V region genes of a hybridoma specific for prostate-specific membrane antigen, the cytoplasmic domain is the cytoplasmic domain of a molecule which functions as a transducer of a mammalian immune response in the presence of a costimulatory factor, and the connector is a region of one or more amino acids disposed between the PSMA-scFv and the cytoplasmic domain, said connector to be of sufficient length to allow both the PSMA-scFv and the cytoplasmic domain to retain function, whereby the fusion receptor is effective when expressed in a T-cell to promote a cellular immune response to prostate-specific membrane antigen.

2. The fusion receptor of claim 1, wherein the cytoplasmic domain comprises a ζ-chain of CD3.

3. The fusion receptor of claim 1, wherein the cytoplasmic domain is derived from CD28.

4. The fusion receptor of claim 3, wherein the cytoplasmic domain is a portion of CD28 cDNA spanning amino acids 336-663.

5. The fusion receptor of claim 1, wherein the cytoplasmic domain is derived from 41-BB.

6. The fusion receptor of any of claims 1 to 5, wherein the connector is a CD8 hinge.

7. A method for treating a patient suffering from cancer, wherein the cells of the cancer or neovasculature associated with the cancer express prostate-specific membrane antigen, comprising the steps of:

(a) preparing an expression vector comprising an expressible polynucleotide molecule encoding a fusion protein in accordance with any of claims 1 to 5;

(b) transducing the expression vector into peripheral blood lymphocytes obtained from the patient to obtain transduced lymphocytes expressing the fusion protein; and

(c) reintroducing the transduced lymphocytes into the patient, whereby said transduced lymphocytes respond to antigen on the surface of the cells of the cancer to generate a cytolytic immune response to the cells of the cancer.

8. The method of claim 7, wherein the expression vector is transduced into the peripheral blood lymphocytes in an *ex vivo* process.

9. The method of claim 7, wherein the expression vector is an SFG vector.

10. The method of claim 9, wherein the expression vector is transduced into patient PBL using gibbon ape leukemia virus envelope-pseudotyped virions.

11. The method of claim 8, wherein the expression vector is transduced into patient PBL using gibbon ape leukemia virus envelope-pseudotyped virions.

12. Peripheral blood lymphocytes transduced with and expressing a fusion receptor in accordance with any of claims 1 to 5.

13. An expression vector comprising a polynucleotide sequence encoding a fusion receptor in accordance with any of claims 1 to 5 and control sequences effective to promote expression of the fusion receptor in mammalian lymphocytes.

14. The vector of claim13, wherein the expression vector is an SFG vector.

15. The vector of claim 14, wherein the expression vector is packaged in gibbon ape leukemia virus envelope-pseudotyped virions.

16. The vector of claim 13, wherein the expression vector is packaged in gibbon ape leukemia virus envelope-pseudotyped virions.

FIG. 1

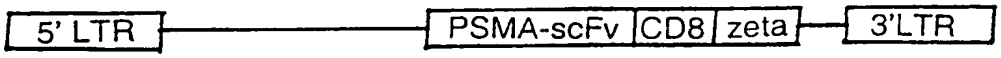


FIG. 3

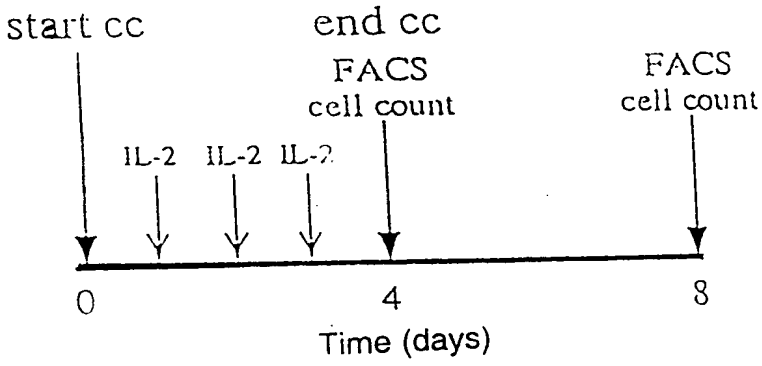


FIG. 5

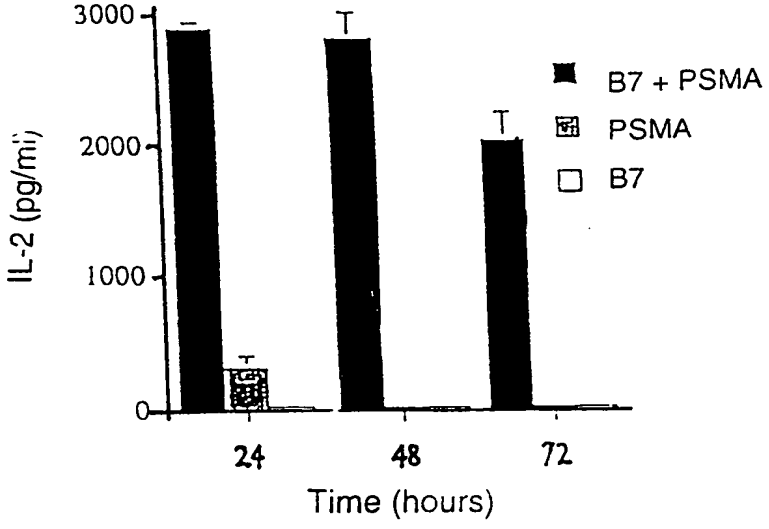
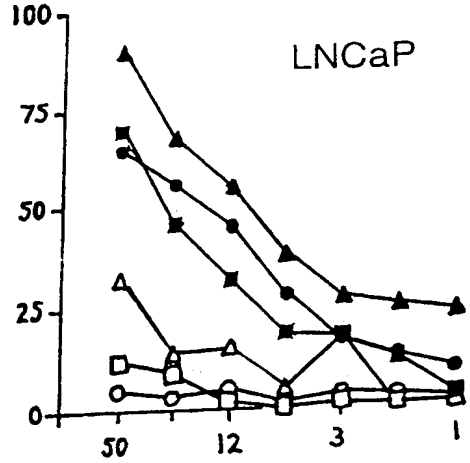


FIG. 2A



	%TR	%CD8+	%CD56+
Pt 1 Pz-1	23	15	<1
Pt 2 Pz-1	40	13	<1
Pt 3 Pz-1	49	44	5
Pt 1 NTP	18	15	ND
Pt 2 NTP	37	13	ND
Pt 3 NTP	27	44	ND

FIG. 2B

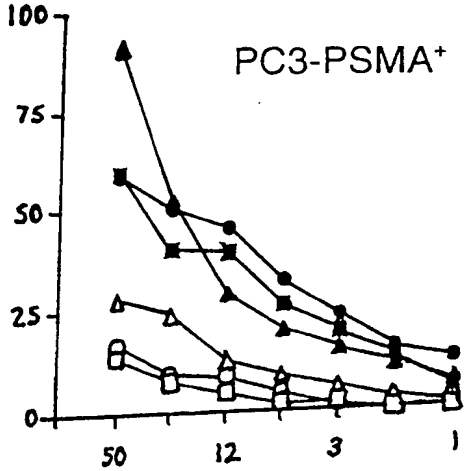
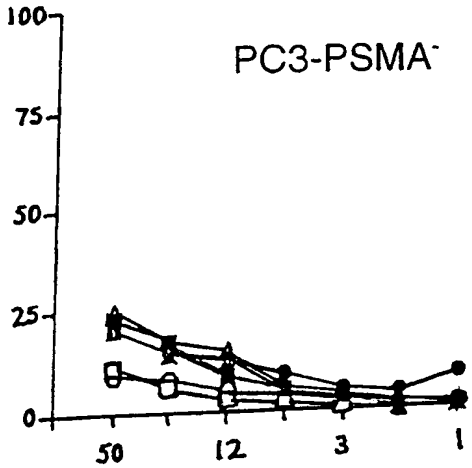


FIG. 2C



% Specific ^{51}Cr Release

FIG. 2D

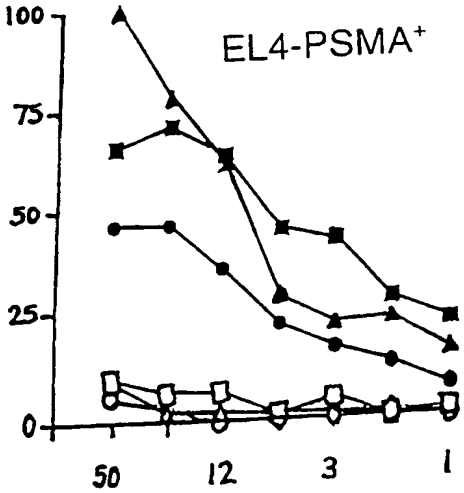
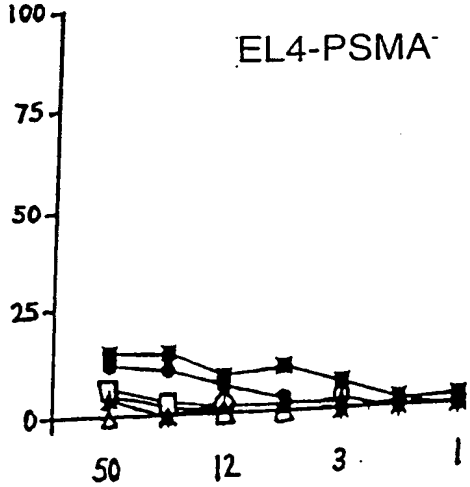


FIG. 2E



E:T Ratio

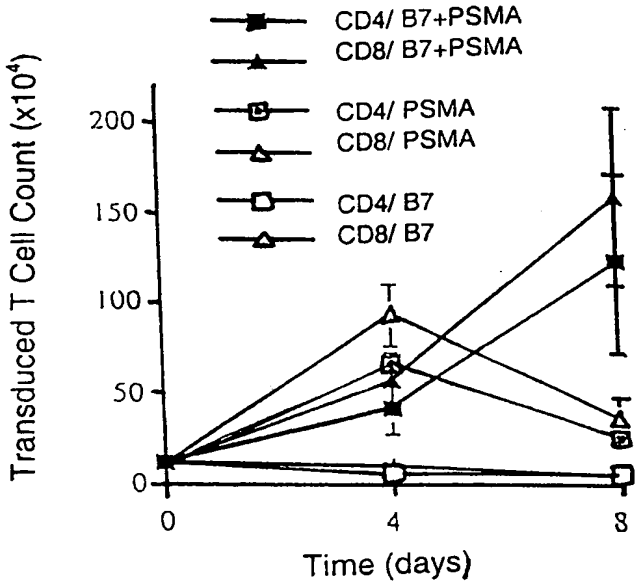

CD4/ B7+PSMA
CD8/ B7+PSMA
CD4/ PSMA
CD8/ PSMA
CD4/ B7
CD8/ B7
Transduced T Cell Count (x10⁴)
200
150
100
50
0
0
4
8
Time (days)

FIG. 4A

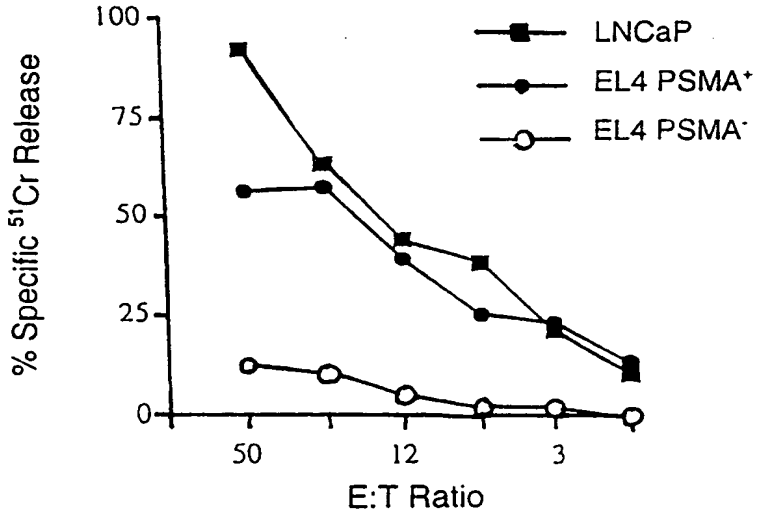

LNCaP
EL4 PSMA⁺
EL4 PSMA⁻
% Specific ⁵¹Cr Release
100
75
50
25
0
50
12
3
E:T Ratio

FIG. 4B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/20349

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 15/63+; A61K 48/00, 39/21, 48/00; C07K 14/00+

US CL : 435/320.1; 514/44; 424/188.1; 424/93.6; 530/324

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/320.1; 514/44; 424/188.1; 424/93.6; 530/324

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN, Caplus, Biosis, Medline, WEST, dialog

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SALGALLER et al. Report of immune monitoring of prostate cancer patients undergoing T-cell therapy using dendritic cells pulsed with HLA-A2-specific peptides from prostate-specific membrane antigen (PSMA). Prostate. May 1998, Vol. 35, No. 2, pages 144-151, see entire document.	1-6, 12-13
Y	MURPHY et al. Phase I clinical trial: T-cell therapy for prostate cancer using autologous dendritic cells pulsed with HLA-A0201-specific peptides from prostate-specific membrane antigen. The Prostate. 1996, Vol. 29, No. 6, pages 371-380, see entire document.	1-6,12-13

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 29 DECEMBER 1999	Date of mailing of the international search report 03 FEB 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer GAI (JENNIFER) MI LEE Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/20349

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LINDEMANN et al. Versatile retrovirus vector systems for regulated gene expression in vitro and in vivo. Mol. Med. July 1997, Vol. 3, No. 7, pages 466-476, see entire document.	7-11,14-16
Y	WO 96/24676 A1 (WHITEHEAD INSTITUTE FOR BIOMEDICAL RESEARCH) 15 August 1996, all (pp. 23,line 22).	7-16
A,E	SIMMONS et al. GM-CSF as a systemic adjuvant in a phase II prostate cancer vaccine trial. Prostate. June 1999, Vol. 39, No. 4, pages 291-297.	1-16
A	LIU et al. Identification of rat prostate steroid-binding protein as a target antigen of experimental autoimmune prostatitis. Implications for prostate cancer therapy. J. Immuno. 1997, Vol. 159, No. 1, pages 472-480.	1-16